

ON VACCINE THERAPY AND IMMUNISATION IN VITRO.

*Based on an Address to the Medical Society of London
on October 27th, 1930*

BY

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ON VACCINE THERAPY AND IMMUNISATION IN VITRO.

THE methods of combating infections in the organism can be divided up into (a) those which aim at removing or extirpating the microbes by mechanical means; (b) those which aim at killing the microbes by the agency of chemical antiseptics; (c) those which make use of the antibacterial powers which already exist in the organism, but do not concern themselves with increasing these; and (d) those which aim at developing increased bactericidal powers in the blood or passively supplementing these powers.

It will be useful to preface what I have to say about vaccine therapy by a brief survey of the alternative methods of treatment.

Methods of *surgical extirpation* may be distinguished into incomplete operations which confine themselves to the removal of a conspicuous focus of infection, and those which aim at the complete eradication of an infection combined with an excision of a *locus minoris resistentiæ*.

Operations of the former kind were only a few years ago extensively employed in surgical tubercle. They were resorted to upon the idea that the organism would, when the full weight of the infection had been taken off, be competent to cope with the germs that were bound to be left behind. This optimism was, of course, more often than not disappointed, the operation being frequently followed by a recrudescence of the infection. This, in point of fact, is just what might have been expected. For operations upon infected foci must always carry with them a risk of producing a negative phase of diminished antibacterial power and favouring the spread of the infection.

Similar dangers attach to enucleation operations of the kind which leave open raw surfaces. Here, if the resistance of the organism is reduced, reinfection from outside is likely to follow.

Treatment by Antiseptics.

The history of antiseptic treatment is the history what has followed from the erroneous assumption that chemical agents which kill microbes when brought

into application in water will do the same when brought into application in serum-impregnated tissues, suppurative discharges, and blood. The ordinary medical man deems that assumption to be somehow right, and he fails to see any reason why it should not be. And he feels sure that if the assumptions he made were wrong, clinical observation would very quickly bring this out.

That confidence is quite misplaced. Probative evidence of the harmfulness of a treatment is furnished only when the clinical condition of a patient becomes conspicuously worse after the application of a treatment, and this happens again and again. Similarly probative evidence of the efficacy of a treatment is furnished only when the clinical condition of a patient is indubitably better after treatment, and this happens repeatedly. In every other case clinical observation leaves us in incertitude as to whether a method of treatment is doing a modicum of good, or a modicum of harm, or is doing neither good nor harm.

Once these inherent limitations of clinical methods are realised it becomes obvious that we must, if medicine is to make steady advance, find some method of distinguishing treatment which is in an inconspicuous manner useful; from treatment which is in an inconspicuous manner harmful; and from treatment which does neither harm nor good.

The first thing to lay to heart here is that it is hopeless to trust in such matters to our unaided senses, and that it is in the case of antiseptics fallacious to draw inferences from the clinical condition of the patient or the condition of a wound. If we seriously want to arrive at the therapeutics of a bacterial infection we must do two things.

First, we must experiment upon simpler biological complexes—complexes such as the blood or the pus or inflammatory fluids derived from a focus of infection, or, better still, upon separated blood fluids, transudation fluids, and leucocytes.

Secondly, we must, in order to discover the event of a therapeutic intervention, have recourse to laboratory technique and scientific apparatus.

Let me give a concrete illustration of the kind of therapeutic problem which I have here in view.

As an incident in an enterprise of research undertaken in 1911, with the aim of alleviating the formidable mortality from pneumonia in the mines of the Transvaal, I and my fellow-workers had, *inter alia*, to inquire into the utility of the internal administration of antiseptics, a method of treatment which was in vogue in some of the mine hospitals.

We had here to elect between two procedures—statistical inquiry into the results of treatment being one method; and inquiry into the effect exerted by antiseptics upon the pneumococcus suspended in serum being the other.

Let us consider what the former method of inquiry would have involved. It would have involved, first, obtaining control over the treatment in the mine hospitals. It would have involved choosing one particular antiseptic and one hard and fast scheme of dosage for all the cases, and imposing this upon all the medical officers. It would have involved further the carrying out of the experiments upon a very large number of cases, and arranging for a corresponding number of similar cases to be left untreated. It would have involved also the keeping of medical histories of all the cases and the verification of the diagnoses by post-mortem examinations of all who died.

Again, and this is a very important consideration in connexion with statistical inquiries into any treatment which is believed in by some observers and dissented from by others (and that was the case here), there is in all such cases a very strong presumption that the treatment in question is in reality not very effective, and that the figures when they shall have been arrived at will exhibit a difference too small to be convincing.

Finally, if the aforementioned considerations do not clearly bar the method of statistical inquiry, one has to consider the length of time and the formidable number of patients which would be required for settling by statistics even a single item in a programme of treatment—for example, the superiority of one antiseptic over another, or of one scheme of dosage over another.

From all this it is apparent that the method of statistical inquiry is utterly impracticable, and that it would, if this were the only method available to us, be necessary to abandon all hope of determining whether a particular treatment is in some degree helpful; or in some measure harmful; or leaves things just as they are. There remains, however, the alternative of recourse to laboratory experimentation.

ON THE APPRAISEMENT OF THE VALUE OF ANTISEPTICS BY LABORATORY METHODS.

The earliest, or among the earliest, experiments on the question as to whether antiseptics can be turned to account in the treatment of bacterial infections, would appear to have been those of Koch. It was

shown by him that though sublimate in a dilution of 1 in 1,000,000 inhibited the growth of anthrax in nutrient gelatin, sublimate in double that concentration does not in any way modify the course of anthrax infection in rabbits. Behring showed that this found its explanation in the fact that a dilution of 1 in 10,000 of sublimate was required to restrain the growth of anthrax in serum. And calculation shows that to achieve such a 1 in 10,000 concentration of sublimate in the blood fluids of a 70-kilo man it would (assuming these fluids to measure 2500 c.cm., and assuming also the full quantum of sublimate administered to come into application) be necessary to administer intravenously 0.25 g. of sublimate—i.e., about grs. 4.

Our experiments were carried out in essentially the same way as those of Behring. Graduated dilutions of antiseptics were made in water, on the one hand, and serum on the other, and then in each case the same quantum of pneumococcus culture was implanted. The details of these experiments are set out in my book entitled *The Treatment of Pneumonia by Drugs and Vaccines* (Constable, London, 1914). The upshot was that while lysol, creosote, and guaiacol—the three antiseptics experimented with—kill the pneumococcus in very high dilutions in water they would, for killing the pneumococcus in the patient's blood, have to be administered intravenously in frankly poisonous doses—in the case of lysol in a dose of over 75 minims and in the case of both creosote and guaiacol in a dose of 15 minims.

We now come to some further points in connexion with the testing the efficacy of antiseptics by *in vitro* experimentation. In particular, it must be realised that while the method of measuring of the bactericidal power of an antiseptic upon microbes implanted into serum is infinitely superior to the method of measuring the bactericidal power upon microbes suspended in water, it is, nevertheless, a method which fails to take into regard many very important physiological considerations.

My friend and fellow-worker, Prof. A. Fleming,¹ has very properly insisted that antiseptics which are destined for use in the blood should be tested upon microbes implanted into blood; and similarly, that antiseptics designed for use in a wound should be tested upon microbes contained in or implanted into pus.

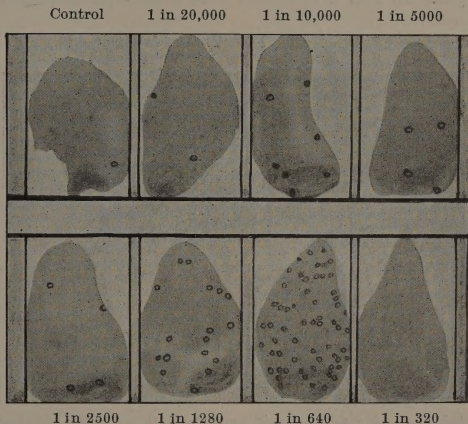
Fleming further pointed out that antiseptics may be usefully tested by implanting staphylococcus into

¹ Proc. Roy. Soc. B., 1924, xcvi., 171.

serum without and with antiseptics and incubating measured quanta of such sera in capillary tubes lined with emigrated leucocytes and, for purposes of control, also in ordinary unlined capillary tubes.

The apparatus and technique employed in these testings having been elsewhere described,² I may deal directly with their results. And those experimental results can be most conveniently shown in the form of diagrammatic pictures.

FIG. 1.



Carbolic Acid. Effect on staphylococci in blood. Equal volumes (25 c.mm.) of infected blood and dilution of antiseptic. 25 c.mm. of blood plated on agar gave 56 colonies.

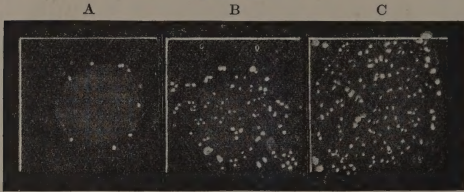
Fig. 1 shows the effect of adding in slide cells an ordinary antiseptic to blood implanted with staphylococci. Infinitely more can be learned from an experiment of this kind than was learned from experiments in which microbes are implanted into serum. The experiments which I made by that method in South Africa taught us only that creosote, guaiacol, and lysol and such antiseptics do not kill microbes immersed in serum. From these of Fleming we learn that antiseptics added to blood in small doses render the blood more congenial to microbial growth, and that

² Most of it in my "Technique of the Teat and Capillary Tube," second edition, Constable, London, 1921.

they, when added in the largest doses that could be used therapeutically, convert the blood into a medium in which serophytic microbes grow unrestrained. (These results are due to the antiseptic entering into destructive chemical combination with the leucocytes.) Finally we learn here, as in experiments such as those we made in Africa, that bactericidal effects are achieved only where frankly poisonous doses are resorted to.

Exactly the same lessons are taught by Fleming's marvellously simple and ingenious experiments with pus. In Fig. 2 it is seen that a non-corrupted pus

FIG. 2.

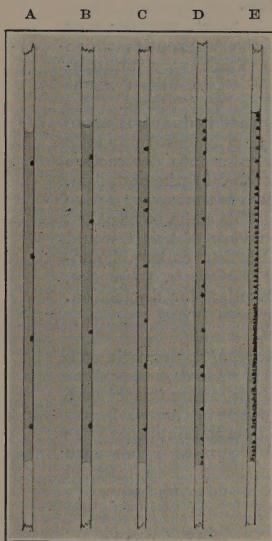


- (A) A drop of wholesome uncorrupted pus obtained from a wound was here implanted upon an agar plate and incubated under a cover-glass. Here all the microbes were killed excepting a few which have grown out into colonies in the film of fluid pressed out from the pus by the weight of the cover-glass.
- (B) A similar drop of pus which had been heated to 47° C.
- (C) A similar drop of pus which had received an addition of 1 in 300 carbolic immediately before it was imposed upon the agar plate.

taken from a wound and kept from drying (by placing it on a moist agar surface under a cover-glass) possess not only sufficient, but (as other experiments show) more bactericidal power than is needed to kill such numbers of microbes as are ordinarily contained in it. And the figure shows that when half per cent. carbolic acid is added to such pus its bactericidal power is forfeited—this being, as in the case of the carbolised blood, due to a poisoning of the leucocytes.

Fig. 3 again enforces the same lesson. Here carbolised and non-carbolised sera are implanted in each case with staphylococcus. In Fig. 3 A where the staphylo-implanted serum has been cultivated in tubes lined with emigrated leucocytes we see that the number of colonies which develop increases as more and more carbolic acid is added—this result being as in the other experiments attributable to a poisoning of the leucocytes by the antiseptic.

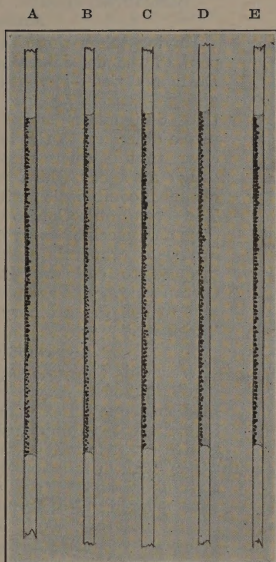
FIG. 3 A.



Leucocyte-lined capillary tubes filled in in each case with serum containing the same charge of staphylococcus and in the case of (B) (C) (D) and (E) an addition of carbolic acid.

- (A) Serum with no carbolic.
- (B) " " 1 in 3200 carbolic.
- (C) " " 1 in 1600 "
- (D) " " 1 in 800 "
- (E) " " 1 in 400 "

FIG. 3 B.



Unlined capillary tubes filled in in each case with the same charge of staphylococcus and in the case of tubes (B) (C) (D) and (E) an addition of carbolic acid.

- (A) Serum with no carbolic.
- (B) " " 1 in 3200 carbolic.
- (C) " " 1 in 1600 "
- (D) " " 1 in 800 "
- (E) " " 1 in 400 "

In Fig. 3 B where precisely the same sera are cultivated in unlined capillary tubes we see that the number of microbes which grow out into colonies is the same in all the tubes. In other words, carbolic acid in strengths up to 1 in 400 fails to inhibit the growth of staphylococcus in serum.

Chemotherapeutic Agents or Endohæmic Antiseptics.

Having taken these things to heart, we may now pass to the consideration of the antiseptics which Ehrlich styled—I venture to think very unfortunately—*chemotherapeutic agents*. Two things about that designation strike me as unfortunate. The prime objection to the term is that it does not make clear that Ehrlich meant by a chemotherapeutic drug: an agent which would enter into destructive chemical combination with microbes in the organism. The second objection to the term is that every active drug and vaccine has an indefeasible claim to be called a chemotherapeutic agent. In view of this I would propose to call the drugs Ehrlich called chemotherapeutic agents *endoserics*, or *endohæmic*, *antiseptics*, and to call the ordinary antiseptics, for distinction, "*ectoseric*."

OPTOCHIN.

With this I resume the account of my experiments made in South Africa in 1911. After finding that medicinal quanta of lysol, creosote, and guaiacol were incompetent to kill pneumococci in blood, I proceeded to make trial of optochin—a quinine derivative which had, in the hands of Morgenroth, given very striking results in the treatment of mice infected with a highly virulent pneumococcus. Morgenroth's results pointing, as it seemed to me, to optochin being an endoserics antiseptic—I commenced operations by adding it in graduated doses to serum implanted with pneumococci. These experiments showed that optochin exerted a bactericidal effect upon pneumococci in serum, and that it exerted this effect in serum and in water indifferently in all dilutions up to 1 in 400,000.

Similar experiments showed that optochin exerts this bactericidal effect specifically upon the pneumococcus. Ordinary ectoserics antiseptics exert their effect upon microbes without distinction of kind.

We further found that the serum of animals and man was bactericidal for the pneumococcus for at least five hours after the ingestion of optochin.

These findings were confirmed by Schiemann ³

³ Zeits. f. Immun. Orig., 1915, xxiv., 167.

in 1915. His experiments showed that the pneumococcus implanted into citrated blood was killed by optochin employed in dilutions as high as 1 in 1,000,000.

There are not sufficient clinical data for deciding definitely upon the value of optochin in the treatment of human pneumonia for, owing to the drug affecting the patient's eyesight, experimentation with it had everywhere to be broken off. The therapeutic effect would, however, appear to have been, taken generally, disappointing.

SALVARSAN.

Let me now deal with another chemotherapeutic agent, to wit, salvarsan, and its application to the treatment of anthrax, streptococcus, and staphylococcus infections.

With regard to the anthrax bacillus, the experiments of Schiemann showed that salvarsan exerts in dilutions such as can be obtained in the blood by the exhibition of a *dosis tolerata* a bactericidal effect upon this microbe. His experiments further showed that it takes with such high dilutions a number of hours to effect the destruction of the anthrax bacillus.

The history of the idea that salvarsan might exert a bactericidal action upon the streptococcus in the body is as follows: That salvarsan might possibly exert a bactericidal effect not only upon the spirochætes of syphilis which were Ehrlich's special target, but also upon the associated streptococcal and staphylococcal infections suggested itself to me in treating cases of rupia with one of the earliest samples of 606. What specially impressed me was the almost magical disappearance of the streptococci from the sequence of cultures made from the lesions of patients under treatment. To test the possibility of this being due to a bactericidal action exerted directly upon the streptococcus I proceeded next to administer 606 to a case of malignant endocarditis. That case, like other cases, ended fatally, but the results were so far favourable that for a time the patient's temperature regularly came down after each injection of 606.

Later on the question as to whether the blood fluids become, after the injection of 606, bactericidal to the staphylococcus, was investigated and answered in the affirmative by experiments made by two of my fellow-workers, S. R. Douglas and L. Colebrook.⁴

Later researches made by Colebrook⁵ in connexion

⁴ THE LANCET, 1916, i., 181.

⁵ Med. Res. Council Spec. Rep. Series, 119.

with the bactericidal effect exerted upon the streptococcus brought out the following facts :—

(1) The variety of streptococcus which is most sensitive to the bactericidal action of salvarsan is the *Streptococcus hæmolyticus*. The others are much less sensitive to the drug administered in medicinal doses.

(2) The blood is, by the intravenous injection of salvarsan, rendered more bactericidal to the *Streptococcus hæmolyticus*. The same result is obtained by adding the drug in similar concentration to the blood in vitro.

These results do not, as was pointed out by Colebrook, mean that salvarsan is not poisonous to leucocytes. It means only that the drug, when employed in medicinal concentrations, is less poisonous to the leucocytes than it is to the streptococcus.

A further important finding which concerns these results must now be noted. Colebrook—like Schiemann, in working with anthrax—found that salvarsan when employed in medicinal doses in serum kills streptococcus only after operating upon them for three hours. Now this means, of course, that salvarsan must, if it is to do any good, continue to circulate in the blood in adequate concentration for that minimum number of hours. Convincing *in vitro* proof that it does so must always be difficult to obtain. What requires in this connexion to be borne in mind is that if a sample of blood drawn off and tested after intravenous injection contains arsenic in effective quantities, that is not in itself conclusive. For the arsenic content in a blood sample which is tested in vitro continues unchanged while the arsenic in the circulating blood may be in steady decline.

It follows from the fact that data with regard to the amount of arsenic in the blood and the rate of elimination in the blood are with difficulty accessible that the utility of salvarsan in the treatment of bacterial infections must in the last instance be determined by clinical experiment.

Favourable results have been reported from the use of the drug in anthrax, and this furnishes ground for hope that success may be obtained also in the treatment of streptococcal infections; and further ground for hope is furnished by the fact that Colebrook, by a course of subcutaneous inoculations of arsenicals, has been able to keep the strepto-bactericidal power of the blood at a high level for several weeks.

Conclusions Relating to Chemotherapy.

Let me now try to summarise the outcome of this, I am afraid, long-drawn discussion. Two things in particular have come out clearly :—

(1) The first is that a bactericidal and sterilising action in the organism can be looked for only from drugs which can

be used in concentrations which exert a bactericidal action in the serum and blood *in vitro*, and it is futile to expect any advantage from the exhibition of drugs which abolish the bactericidal action of leucocytes.

(2) It is likewise futile to expect a bactericidal and sterilising effect in the organism from the exhibition of such endohæmic antiseptics as are both slow in action and vanish very quickly from the circulation.

In obedience to these principles we must cross off the list of intravital endohæmic antiseptics, not only all the ordinary familiar antiseptics, but also a large number of drugs which are or have been reputed chemotherapeutic agents.

We must cross off to begin with *sanocrysin* which was, in despite of the fact that it fails to kill the tubercle bacillus when used in a 1 in 500 concentration⁶ in serum, bruited abroad as a chemotherapeutic remedy for the treatment of tuberculosis. We must further, despite the fact that *optochin* kills the pneumococcus in the blood in very high dilutions, cross it off our list for the reason that it is gravely poisonous. Further, we must cross off *mercurochrome*, for this drug added to serum and blood in the highest concentrations which would be permissible in the organism fails to exert a bactericidal effect.⁷ We must further cross off from the list of chemotherapeutic agents *flavine* and *gentian violet*, for these dyestuffs, though they are both endohæmic antiseptics, are eminently slow in their action, and vanish very quickly from the circulation.

If we question ourselves what remains of the chemotherapy of bacterial diseases proper when all the above items have been crossed out, our answer must be that apparently only salvarsan and its congeners remain. We have seen with regard to these that they can increase the bactericidal power of the blood to the anthrax bacillus and the *Streptococcus hæmolyticus*; and that they may perhaps remain in the circulation in sufficient concentration long enough.

This general conclusion is very important when we ask ourselves exactly how much can be done by methods other than vaccine therapy in the treatment of bacterial infections.

Kataphylaxis.

Before I pass on to consider what can be done by vaccine therapy it will be well perhaps to gauge what can be done by other procedures which aim at killing microbes by what I have ventured to call *kataphylactic* measures, that is measures which determine

⁶ Brit. Jour. Exp. Path., 1926, vii., 174.

⁷ Unpublished Experiments of Colebrook and Fleming.

to the focus of infection the bactericidal powers resident in the blood fluids and leucocytes. Examples of what I have called kataphylactic measures are the opening up of abscesses, the evacuation of infected discharges, the application of Bier's bandages and of hot compresses, all kinds of radio- and helio-therapy, and the drawing out of corrupted serum from an infected surface by the agency of hypertonic salt solution. These methods are in every case of service. But when all is said and done the degree to which they are useful in the particular case depends upon the antibacterial potency of the blood—this being the reservoir of antibacterial power which every kataphylactic measure draws upon. This may be illustrated by the fact that the opening up of a localised tuberculous focus when the bactericidal power of the blood is below par does very little towards extirpating the infection while such a measure is more effective when the antituberculous power of the blood is well above par.

Principles of Vaccine Therapy.

I pass now to the subject matter proper of this discussion—the employment of vaccines for the purpose of enhancing the antibacterial power of the blood in infected patients.

Let me—for it is axiomatic that every therapeutic measure should repose upon a sound theoretical basis—begin what I have to say about vaccine therapy by harking back to its intellectual foundations, and justifying principles. The principle of vaccine therapy is the same as that of Pasteur's antirabic inoculations. Pasteur gave intellectual countenance to his antirabic inoculations by pointing out that a man who has been bitten by a rabid animal may in the interval between the implantation of the virus and the development of overt symptoms be quite properly classed as uninfected, and that he may consequently, with propriety, be preventively inoculated.

That was the principle to which I gave extended application by pointing out that all localised infections may be conceived of as precursors (generally, of course, abortive precursors) of septicæmic infections. And the way I pictured the situation to myself was that, so long as an infection remains localised, the infected tissues combat the infection unaided, the uninfected tissues remaining in the meanwhile impassive and idle. Picturing things to myself in that way led inevitably to the thought that it would be possible by the aid of vaccines "to turn to account in the interest of the infected tissues the unexploited immunising faculties of the uninfected tissues."

In formulating that principle I had in view under the denomination of "tissues," the "fixed tissues." I meant, so far as I meant anything precisely, the subcutaneous tissues into which the vaccine was inoculated and the various cellular organs with which the vaccine would, after absorption, come into contact. I would now propose to replace the term tissues in the above formula by the term leucocytes. That proposed change of wording is dictated by the consideration that conclusive evidence (some of this I propose to adduce) has been obtained of the elaboration of antibacterial substances by leucocytes, while we have, despite every sort of search, failed to find any indication of the generation of antibodies by the fixed tissues.

With that I pass to another point. When it is claimed for therapeutic procedure that it will enhance the antibacterial power of the blood, that contention should in every case—I need hardly say how very rarely this is even attempted—be confirmed by evidence obtained by laboratory methods. To comply in the case of vaccine therapy with this requirement it was necessary to devise new methods of blood testing. This was necessary because the method of testing the bactericidal power of the serum which I had devised for use in connexion with anti-typhoid inoculation is inapplicable where we are dealing with serophytic microbes, such as the staphylococcus and streptococcus.

The Opsonic Index.

It was to meet this new requirement my late friend and fellow-worker, W. B. Leishman, devised his method for measuring the phagocytic power of the blood. And the method for measuring the opsonic power of the serum, which was an outcome of Leishman's method, was also devised in the service of vaccine therapy.

Without these methods it would, except only in cases in which inoculation produces dramatic improvement or dramatic exacerbation of symptoms, have been impossible to decide whether a dose of vaccine was doing good or harm, whether it should be increased or diminished, and after what interval inoculation should be repeated.

But the guidance furnished in treatment of individual cases was not, as a matter of fact, the chief intellectual profit gained from our measurements of the opsonic index. (We made for many years 30,000 to 50,000 of such measurements annually at St. Mary's.) We learned from those measurements

principles that seem to me to lie at the very foundations of medicine. Let me enumerate them.

(1) To begin with, we learned from these measurements that a succession of negative and positive phases such as I had described⁸ in connexion with typhoid vaccination occurred when any vaccine was inoculated in quantity sufficient to produce appreciable constitutional disturbance. With regard to the negative phases, I had already in connexion with prophylactic inoculation pointed out that a negative phase means a temporarily lowered resistance to bacterial attack, and that, therefore, a negative phase should never be induced in a patient who is living in infected surroundings. The same principle holds *a fortiori* of a patient who is already the subject of an infection. Here the induction of a negative phase may lead to an exacerbation and a dissemination of the infection.

(2) The second general principle which was learned from measurements of the opsonic index (and Dr. John Freeman took a conspicuous part in the initiation and development of this research) was that *auto-inoculation* (the term had to be invented) can be artificially evoked by a large variety of procedures, and may also occur automatically. Such auto-inoculations produce negative and positive phases in exactly the same way as inoculations of vaccine.

We further learned in connexion with auto-inoculations that they can be produced in patients by active and passive movements, and by sending an ampler lymph stream through an infected focus (the so-called *stagnation method* of Bier). Further, that they can be produced by helio-therapy, radio-therapy, and the application of heat. And we learned also, quite early in our investigations, that automatic auto-inoculations occur whenever a patient who is the subject of an extensive infection is allowed out of bed. Finally, it was in this connexion brought home to us that in a progressive infection such as phthisis—the same applies, of course, to all acute infections—there sooner or later supervenes a stage when the biography of the patient—even when he is kept completely at rest—resolves itself into a succession of spontaneous auto-inoculations. It is the signal merit of Dr. Marcus Patterson to have applied these principles to practice in the treatment of phthisis, and to have realised that success depends largely upon the regulation of the auto-inoculations by the

⁸ Treatise on Antityphoid Inoculation. Constable, London, 1904.

regulation of exercise, and to have appreciated further that the most rapid and effective remedial treatment for pyrexia produced excessive auto-inoculations is absolute immobility in bed.

(3) A third advantage—and this was, of course, a question of purely practical as distinguished from theoretical importance—gained from our innumerable measurements of the opsonic index undertaken before and after inoculations undertaken upon patients, was that we learned what quanta of the various vaccines can be administered to slightly infected and non-pyrexial patients without risk of inducing a negative phase.

(4) The fourth advantage gained from our measurement of the opsonic indices of patients was that through these there was formulated a general schema or principle of dosage. The all-important principle which was arrived at is that the dose of vaccine administered to a patient should be inversely as the number of microbes affecting (i.e., delivering antigen) into his circulating blood. In other words, we should in constitutional infections give only minimal doses of vaccine; should give intermediate doses to patients suffering from a strictly limited local infection; and should exploit large doses which produce negative phases only in healthy patients and for prophylaxis.

OPSONINS AND PHAGOCYTOSIS.

Having shown what important principles were learned from our measurements of the opsonic power of the blood-serum, I turn to the question whether we have in the opsonic index a true measurement of the phagocytic power of the blood.

My original idea—based upon investigations carried out with S. R. Douglas⁹—was that the opsonic index furnished a true measure of the phagocytic power of the blood. That conclusion was arrived at by taking from our out-patients (these were non-pyrexial and, therefore, probably non-auto-inoculating patients) bloods with high and bloods with low opsonic indices, and comparing the leucocytes of these bloods with those of our own bloods. We employed for that purpose what I have since called the *chiastic procedure*. That procedure consists of digesting with a microbic suspension: (1) the patient's washed corpuscles used in conjunction with his own serum; (2) his corpuscles used in conjunction with a normal serum; (3) washed normal corpuscles used in conjunction with normal serum; and (4) these corpuscles used with the

⁹ Proc. Roy. Soc., 1903, lxxii., and 1904, lxxiii.

patient's serum. The event of these operations carried out on our out-patients showed that in their case the amount of phagocytosis was determined almost entirely by the serum, and hardly at all by the corpuscles employed in the phagocytic mixture.

Afterwards S. G. Shattock and L. Dudgeon corrected these observations. They employed melanin granules instead of microbes, and carried out the tests upon pyrexia and auto-inoculating, instead as we had done upon patients who were non-pyrexia and, presumably, non-auto-inoculating patients. Shattock and Dudgeon's¹⁰ investigations clearly demonstrated that the phagocytic avidity of the leucocyte is a variable factor, and that the phagocytic avidity of a patient's leucocytes may sometimes be much greater and sometimes much less than that of the normal leucocytes. Their investigations—their results were, as already mentioned, obtained with melanin granules—further established that phagocytic efficiency is a non-specific function of the leucocyte. These fundamentally important observations show that opsonic power is, at any rate, in pyrexia cases, a fallacious measure of the phagocytic power of the blood.

We have in the fact that the phagocytic avidity of leucocytes may suffer change a possible explanation of our having often found very high opsonic power in patients dying of phthisis. Examination might have shown these high indices to have been counterpoised by diminished phagocytic avidity. For in following up the observations of Shattock and Dudgeon we have found that the general rule in severe bacterial toxæmia is for the efficiency of the patient's leucocytes to be low while his opsonic index is high. It is easy to see a possible causal connexion between these two findings. The leucocytes may deplete themselves of antibacterial elements by parting with these to the blood fluids.

I now pass to an issue even more fundamental than that which was raised by Shattock and Dudgeon. We may ask ourselves whether a measurement of the phagocytic power furnishes in any case an adequate measure of the antibacterial power of the blood. That question must be answered in the negative. And our answer must be in the negative—*first*, because the leucocytes can kill bacteria not only intracellularly, but also extracellularly, by excreting bactericidal elements into the surrounding fluids; and *secondly* (and Douglas¹¹ has examined this question

¹⁰ Proc. Roy. Soc. B., 1908, lxxx., 165.

¹¹ Proc. Roy. Soc. B., 1916, lxxxix.

in detail), because phagocytosed microbes may fail to be digested in the leucocytes of one blood while they are in the leucocytes of another blood rapidly digested.

It follows that we cannot, if we wish to arrive at a true measure of the antibacterial power of the blood, content ourselves with measuring phagocytic power. We must measure the actual bactericidal effect exerted.

Antibacterial Power of Blood.

I accordingly set myself to devise methods for measuring, first, the hæmobactericidal power exerted by the blood and its population of leucocytes upon serophytic organisms such as the staphylococcus, streptococcus, pneumococcus, and the tubercle bacillus. The problem I set myself here was to find, not a method of explantation to tell us what number of serophytic microbes are killed off without residue, but a method of inculturing to tell us what percentage of enumerated microbes implanted would grow out into colonies in the blood. The reason why the one and not the other kind of method is required to measure the effect exerted by the blood by serophytes will presently be apparent.

The slide cell method which makes use of defibrinated blood was one of the methods of inculturing devised for this purpose. It is illustrated in Figs. 1 and 4, and is one of the methods put to use by Fleming in his experiments on the action of antiseptics.

A second method devised for the same purpose is the following :

Graduated implantations of microbes are made into uncoagulated instead of into defibrinated blood, and the implanted blood volumes are then drawn up into capillary tubes. After overnight incubation the capillary clots are blown out, are treated with 1 per cent. saponin, and are then hung up in water until their colour is completely discharged. They are then dried and stained, and the colonies are enumerated under the microscope.

This, of course, gives—in particular in the case when the colonies are close-packed—a more accurate enumeration than the naked-eye observation which we trust to in the slide-cell method. It also gives us indications as to the rôle which phagocytosis plays in the killing of microbes. For the colonies found in the clots can be ranged into three classes: *naked colonies*—i.e., those which are not surrounded by leucocytes; *ringed colonies*, colonies surrounded with leucocytes; and what we may call blind, blank, or *obliterated colonies*—i.e., aggregations of leucocytes surrounding a presumably extirpated bacterial colony.

A third method—and this differs from the second only in the mode of implantation—is the “wash and afterwash” method. Here we draw up into a long capillary tube, first as a forerunner, a diluted bacterial culture, and then follow on with 10 equal volumes of blood separated off in each case by a bubble of air.

I will now relate what these methods have taught us about immunisation and the killing of serophytic microbes by the blood. Let me first explain the following points :

To begin with, the normal serum constitutes a very excellent culture medium for the class of microbes we are here considering. The same holds of the plasma. This, since the plasma is always full of blood-platelets, means incidentally that these formed elements also can here be dismissed from consideration. Further, the red corpuscles can, in appraising the bactericidal powers of the blood, be neglected. That follows by the fact that deleucocytized blood—i.e., the mixture of red corpuscles and serum which is obtained by passing blood through a close filter¹²—is as good a culture medium for serophytes as clear serum. It follows from this galaxy of premises that the bactericidal action which is exerted by a blood upon serophytic microbes is neither more nor less than the bactericidal action exerted by its population of leucocytes.

The next issue which comes up in connexion with the killing of serophytes by leucocytes is that as to whether these microbes are killed exclusively by phagocytosis followed by intracellular digestion ; or whether they are killed also, and if so to what extent, extracellularly by bactericidal elements elaborated by the leucocytes and excreted by them into the serum.

Important data which have, as we shall see later, a bearing upon this question, are furnished by a study of the event of adding graduated quanta of serophytic microbes to equal volumes of blood.

FUNDAMENTAL EXPERIMENTS.

I have already published experiments bearing on this question. But it will be convenient to give a concrete illustration of what happens when my blood (it is a blood of somewhat low staphylobactericidal power) is implanted with different quanta of staphylococcus. I subjoin protocols of

¹² Wright, A. E. : *THE LANCET*, 1926, i., 4.; Fleming, A. : *Brit. Jour. Exper. Path.*, 1926, vii., 281.

two typical experiments confining myself for the moment to experiments carried out in slide cells.

Expt. 27.7.22.—Forty c.mm. volumes of A. E. W.'s defibrinated blood implanted with different quanta of staphylococcus culture and cultured in slide cells. (Table I.)

TABLE I.

Approximate number of staphylococci implanted.*		No. of colonies which developed.	Percentage of microbes that survived.
Per c.cm.	Per 40 c.mm.		
6,400	256	95 } 95½	39
3,200	128	57 } 55	43
1,600	64	53 } 30	47
800	32	34 } 15	46
400	16	26 } 11½	72

* The number of microbes implanted was here and in all the experiments which follow deduced from the number of microbes which grew out into colonies in serum.

Expt. 1.8.22.—Graduated dilutions of staphylococcus culture were implanted into measured volumes of A. E. W.'s blood. The blood was then tested by two observers. (Table II.)

TABLE II.

Observer A. E. W.

Implantation per c.cm. of blood.	No. of microbes implanted into 40 c.mm. of blood.	No. of microbes which developed into colonies.	Percentage of survivors.
8,400	336	64	19
4,200	168	28	17
2,100	84	24	28
1,050	42	7.5	18
525	21	11.5	55
262	10½	5	50
131	5	5	140
66	3	7	
33	1.5	1.5	
	671	153	22.8

Observer L. C.

9,600	384	74	19
4,800	192	44	23
2,400	96	27	28
1,200	48	13	27
600	24	15	63
300	12	6	50
150	6	5	114
75	3	5	
38	1.5	2	
	766	191	24.9

The explanation of the fact that the implanted microbes are here in no instance killed off without residue is furnished by the consideration that extra-cellular killing involves close proximity, and phagocytosis actual contact, between the microbes and leucocytes. In connexion with these requisites attention must be given to the following series of facts: (1) there are always in the blood large areas unoccupied by leucocytes (the volume of the contained leucocytes stands to the volume of the containing blood in the ratio of about 1 to 300); (2) these regions are for the major part leucocytically unpatrolled or very ineffectively patrolled; and (3) since it can, by appropriate experiments, be shown that leucocytes are insensitive to the feeble chemotatic stimuli which emanate from single microbes, the finding of isolated leucocytes by microbes must—even in patrolled areas in the blood—be a matter of chance. In other words, it depends upon the microbe happening to lie directly in the path taken by the leucocyte.

The fact that with larger and larger implantations larger and larger numbers of microbes are killed, shows that the blood develops under the impulsion of microbial stimulation increased bactericidal power.

INTERPRETATION OF THE DATA.

From this then follows the important principle that our methods of hæmobactericidal measurement are not in the same category as quantitative chemical tests. They are, in reality, biological tests; tests which tell us not what amount of bactericidal energy is actually resident in a sample of blood, but what bactericidal energy can, under the impulsion of microbial stimulation, be developed in that blood. In other words, they are comparable to tests made to find out what amount of muscular power can, under stimulation, be put forth by a muscle.

It is pertinent to note in passing that the general principle of which we have illustration here may hold true, not only of bactericidal, but also of opsonic power. It is conceivable that the values for opsonic power which are registered when microbes are incubated with a mixture of serum and leucocytes may represent, not as hitherto assumed, the native opsonic power of the serum, but native opsonic power supplemented by opsonins excreted into the serum by leucocytes under the impulsion of a microbial stimulus. Experimental data which bear upon this question will come up for consideration later. For the moment it will suffice to point out that by centrifugalising instead

of incubating a phagocytic mixture, and by conducting our phagocytic operations at the temperature of the air instead of at blood heat, we can reduce the exposure of the leucocytes to microbial stimulation to a minimum.¹³

The Immunising Response.

Now that our measurements of the bactericidal power of the blood have brought us face to face with the conception of immunising response to the inoculation of microbes into the blood *in vitro* it will be appropriate to explain the genesis of that idea.

My first experiments on the question of immunising response made by the blood were prompted by the fact already mentioned that we had, despite strenuous experimental search, failed to find any indication of the production of antibacterial substances by the fixed tissues. In view of that I performed the following experiment :—

Having placed double ligatures loosely round the jugular vein of a rabbit and having tied off all tributaries, vaccine was introduced into the vein of the opposite ear, and thereupon the double ligatures were drawn tight so as to isolate a portion of the vaccinated blood within the jugular vein. At the same time a sample of the vaccinated blood was withdrawn from the carotid and was placed in the incubator.

Experiments of this kind showed that the antibacterial response was obtained, not only in the circulating blood, but also in the blood isolated in the vein; and in addition in the blood which was isolated and incubated *in vitro*.

These experiments were followed by others in which graduated doses of a staphylococcus and also of a streptococcus vaccine were added to my own blood *in vitro*. The inoculated samples of blood were then incubated for six hours and afterwards the sera were pipetted off, and then using the "wash and afterwash" procedure 10 volumes of each serum were implanted with a mixture of staphylococci and streptococci. The separate volumes of serum in question were then, after overnight incubation, explanted upon agar. These experiments showed (a) that the serum of blood implanted with an appropriate dose of a staphylococcus or streptococcus vaccine becomes bactericidal to both species of microbe; and, further (b) that the sera of the bloods implanted with an overdose of vaccine furnish, as compared with the serum of the uninoculated blood, a more favourable culture medium for both kinds of microbe.

¹³ Fleming: On Wright's Centrifuge Method of Estimating Phagocytosis, *Brit. Exp. Path.*, 1927, viii., 50.

The next important step undertaken—but this had to wait until the slide cell and other hæmobactericidal methods had been devised—was to investigate, as we have just done, the effect of adding graduated quanta of serophytic microbes to the blood.

IMMUNISING RESPONSE IN VITRO.

I have now, for 12 and more years, experimented continuously upon the immunising response of blood in vitro.

In addition to examining for augmented and reduced bactericidal power in the blood taken as a whole, I have analysed the results which relate to this by chiasitic bactericidal experiments. Further, I have searched for augmented and reduced bactericidal power in the serum—I use here the term reduced as well as the term augmented for the reason that the normal serum, though it is as good a culture medium for staphylococci as nutrient broth, furnishes, there is reason to think, less than 100 colonies for 100 implanted staphylococci. I have further looked for increased and decreased phagocytic response; for increased and diminished opsonic power; and for negative and positive changes in the phagocytic avidity of the leucocytes—keeping in view all the time the problem of the rôle played by each of these items in the general bactericidal power of the blood.

I have employed also as antigens, both living and dead, and in some cases, autolysed microbes, testing the resulting immunising response upon both homologous and heterologous cultures.

Moreover, I have experimented upon the blood before clotting, upon coagulated blood, upon defibrinated blood, and upon isolated leucocytes—either leucocytes which had emigrated from the blood, or leucocytes separated by centrifugalisation.

Again, I have brought living or dead vaccines into operation sometimes by incorporating them into the blood; and at other times by placing a blood containing vaccine upon or side by side with a blood containing a test-quantum of microbes (*Method of Welded Clots*); and, finally, I have—and this is also an effective method of vaccination—inoculated the leucocytes intracellularly. The procedure here in question (I call it the '*baiting method*') consists in taking two samples of blood, giving the leucocytes in one sample, a somewhat sparse phagocytic meal of microbes; and then supplying, after an interval of incubation, both the fed and the unfed populations of leucocytes with a full phagocytic test-meal.

In view of the wide domain covered by these experiments, I must confine myself to setting out here only the most important experimental findings.

Let me begin with the results of the chiasitic experiments.

Expt. I., 29.8.23.—Simple bactericidal experiment in slide cells and also chiasitic experiment. (Observer—L. C.). (Tables III. and IV.).

Female student inoculated with 1000 million staphylococci subcutaneously. Blood A taken before the inoculation; Blood B four hours after. Both bloods were tested with three different suspensions of staphylococcus—2.5 c.mm. of each being added in each case to 50 c.mm. of blood.

TABLE III.—*Hæmobactericidal Experiment.*

Tests of samples of blood before and after inoculation in vivo.

No. of staphylococci implanted	747	249	83
	No. of colonies which developed.		
Blood A	13	5	1
Blood B	4	4	2

TABLE IV.—*Chiasitic Slide Cell Bactericidal Tests.*

No. of staphylococci implanted	420	140
	No. of colonies which developed.	
Corpuscles of Blood—		
(A) with serum of Blood (A)	27	11
(B) „ „ (B)	9	2.5
(A) „ „ (B)	16.5	9.5
(B) „ „ (A)	8.5	5.0

Blood B.—Index of hæmobactericidal efficiency 3.3; index of the bactericidal efficiency of the serum, 1.45; index of bactericidal efficiency of the leucocytes, 2.8.

TABLE V.—*Chiasitic Slide Cell Bactericidal Tests.*

No. of staphylococci implanted ..	736	368	184	92	66
	No of colonies which developed.				Total.
Serum and { Normal blood ..	25	12	5	4	46
corpuscles of { Vaccinated „ ..	36	19	9	7	71
Serum of normal and corpuscles of	54	20	15	6	95
vaccinated blood					
Serum of vaccinated and corpuscles	12	6	3	2	23
of normal blood					

Vaccinated Blood.—Index of hæmobactericidal efficiency, 0.64; index of the bactericidal efficiency of the serum, 2; index of the bactericidal efficiency of the leucocytes, 0.5.

Expt. II.—Inoculation in vitro.—A 100 c.cm. volume of R. T. M.'s unvaccinated defibrinated blood, and also a 100 c.mm. volume of his blood vaccinated with 1,000,000 of dead staphylococci per c.cm. The bloods were then incubated for one hour. After that the sera were pipetted off and the corpuscles washed in saline. Then in each case one volume of washed corpuscles was mixed with one volume of serum, and then 50 c.mm. volumes of each mixture were implanted with one-twentieth volume of graduated dilutions of staphylococcus culture. The implanted bloods were then cultivated in slide cells. (Table V.).

The results, you will see, of these experiments are inconclusive as to the relative importance of the part played by the leucocytes and the serum in the general bactericidal effect.

I must therefore pass on to other experiments. And let me, in order that these may be followed properly, point out that the number of serophytic microbes killed by the blood is determined largely by the disposition of the population of leucocytes relative to the population of microbes, and this disposition is, of course, modified by every physical force that comes into operation upon the blood.

The forces which here require to be considered are : (a) the force of gravity ; (b) the compressional force exerted by the contraction of the clot ; (c) the effect of centrifugalisation respectively upon uncoagulated, defibrinated, and coagulated blood ; and (d) the spontaneous movements of the leucocytes.

With regard to the effect of gravity this comes into consideration in particular when we are operating with defibrinated blood. For here the red corpuscles and leucocytes settle to the bottom ; and the microbes are left floating in the supernatant serum. They are there safe from phagocytic attack, and can, if the serum continues to be non-bactericidal there develop into colonies.

The spontaneous contraction of the clot influences the results by bringing closer together the leucocytes and microbes which were in the unclotted and freshly clotted blood comparatively far apart. In connexion with this it will be recalled that half the volume of blood consists of fluid—and that this in the unretracted clot forms pools in which the microbes are, as in the supernatant serum of defibrinated blood, safe from phagocytic attack. The degree to which an individual clot is compacted by the spontaneous retraction of the fibrin meshwork depends, of course, upon all sorts of variable and incalculable factors.

Centrifugalisation—we may consider first the centrifuging of uncoagulated or defibrinated blood—together alters the distribution of the leucocytes

in those bloods. When uncoagulated blood is centrifuged the large mononuclear leucocytes and the lymphocytes are brought to the surface, and are with a proportion of the blood-platelets agglomerated into a leucocytic cap. Most of the polynuclear leucocytes are assembled immediately underneath. When defibrinated blood is centrifuged for a long period (let us say, a quarter of an hour) in a high speed centrifuge, the polynuclear leucocytes are brought to the top. When microbes are implanted into serum or plasma or are implanted into blood which does not make immunising response, the microbes are not carried down in full proportion by ordinary centrifuging. Many are left in suspension in the supernatant fluid.

When a coagulated blood is centrifuged the clot is, of course, compacted, the lacunæ are abolished, and in the case of an implanted blood the microbes and leucocytes are brought into much closer proximity than in the uncompacted clot.

The results obtained by the centrifugalisation of implanted capillary clots furnish a striking manifestation of the influencing of the hæmobactericidal effect by mechanical factors.

TABLE VI.

No. of staphylococci implanted	520	1560
	No. of colonies which developed.	
Defibrinated blood in slide cells	30 } 52 } 43 43 } 46 }	93 } 121 } 110 114 } 111 }
Unpacked capillary blood-clots	48 } 40 } 44 4 }	124 } 129 } 126½ 11 }
Packed capillary blood-clots. .	4 } 3 } 3½	11 } 48 } 29½

Expt. 7.11.28. Comparison of Packed and Unpacked Blood and Slide Cells.—Four 50 c.mm. volumes of R. M. F.'s blood implanted with an 1800-fold and four others with a 600-fold dilution of staphylococcus, 2.5 c.mm. of staphylococcus dilution being added to each 50 c.mm. of blood. The implanted bloods were then filled into capillary tubes and were kept in the incubator for six minutes to allow time for clotting and for the separation of the clot. Then two tubes from each set of four were centrifuged for two minutes at full speed in the electric centrifuge. All the capillary tubes were then incubated for 18 hours. The clots were then decolorised and the anaerobic colonies in them were counted.

Four 50 c.mm. volumes of defibrinated blood to which the same addition of staphylococcus had been made were then

cultured in slide cells. Higher dilutions of staphylococcus were enumerated in slide cells in watered blood. (Table VI.)

Expt. 16.11.27.—Similar experiment and technique to above. (Table VII.)

TABLE VII.

—	Volume of staphylococcus implanted.		
	$\frac{1}{15}$ Wash.	$\frac{1}{3}$ Wash.	$\frac{1}{3}$ Wash.
	No. of colonies which developed.		
Defibrinated blood in slide cells	48 } 38 } 43	113 } 108 } 111	∞ } ∞ } ∞
Unpacked capillary blood-clots	15 } 13 } 14	39 } 65 } 52	149 } 225 } 187
Packed capillary blood-clots ..	0 } 2 } 1	0 } 0 } 0	32 } 16 } 24

Having got these preliminary matters out of our way we may consider the outcome of repeating the fundamental experiments, varying however the technique, in using here for our implantation instead of defibrinated blood or blood fresh from the circulation, leucocytes derived from centrifuged blood suspended in serum. The following protocols of experiments show that we obtain when we operate with leucocytic suspensions exactly the same results as when we operate with the whole blood.

TABLE VIII.

Dilution of staphylococcus implanted.	No. of colonies which developed in a leucocyte-free serum.	No. of colonies which developed in the leucocytic suspension.	Percentage of survivors.
Staph. 100,000	8 } 8	10 } 9	100
Staph. 50,000	16 } 17 } 16.5	2 } 2 } 2	12
Staph. 25,000	33	6 } 4 } 5	15.5

Expt. 31.10.30.—A. E. W.'s blood. Citrated blood was prepared by adding $\frac{1}{2}$ per cent. of citrate of soda to 50 c.mm. volumes of blood. The blood samples were then centrifuged in capillary tubes and the plasma was pipetted off. The capillary tubes were now cut across immediately below the leucocytic cap and the agglomerated leucocytes dispersed in 30 c.mm. of serum. These serum suspensions of leucocytes were then implanted with, in each case, 5 c.mm. of a diluted staphylococcus culture, and were then incubated in capillary

tubes disposed nearly horizontally. Similar quanta of staphylococcus were, for purposes of enumeration, implanted into leucocyte-free serum. (Table VIII.)

Expt. 7.11.30.—50 c.mm. volumes of R. H.'s blood were centrifuged after the addition of $\frac{1}{2}$ per cent. citrate of soda. The plasma having been pipetted off the leucocytes composing the leucocytic cap were dispersed in 30 c.mm. of serum, and these suspensions were implanted with one-tenth of their volume of graduated dilutions of staphylococcus, and were then incubated in capillary tubes disposed nearly horizontally. (Table IX.)

TABLE IX.

Dilution of staphylococcus implanted.	No. of microbes implanted into each volume of leucocytic suspension.	No. of colonies which developed in each tube.	Percentage of survivors.
Staph. 200,000	} 25	1 } 1	4
Staph. 100,000	} 50	0 } 0.5	1
Staph. 50,000	} 100	1 } 1.5	1.5
Staph. 2500	} 2000	9 } 8 7 }	0.4

Expt. 5.11.30.—R. T. M.'s blood. Leucocytic suspension made by suspending the leucocytes from 50 c.mm. of centrifuged blood in 15 c.mm. of serum.

TABLE X.

No of microbes implanted.	No. of colonies which developed.	Percentage of survivors.
36	9 } 10 $\frac{1}{2}$ 12 }	28
72	20 } 19 18 }	26
144	18 } 18 18 }	12
288	58 } 44 30 }	15
576	60 } 59 58 }	10

Expt. 3.12.30. R. M. F.'s blood.—Graduated implantations of staphylococcus into a weak leucocytic suspension.

Leucocytic suspension prepared in bulk from R. M. F.'s citrated blood and diluted with serum to one-eighth standard; $2\frac{1}{2}$ c.mm. volumes of staphylococcus dilutions were then implanted into 20 c.mm. of this leucocytic suspension—in triplicate and the suspensions were then incubated as above. Five counts made of 1/30,000 dilution in normal serum. (Table XI.)

TABLE XI.

Dilution of staphylococcus.	Count.	Calculated implant.	Colonies developed.	Percentage of survivors.
Staph. 1000	—	720	246 } 259	36
Staph. 3000	—	240	72 } 87	36
			90 }	
Staph. 10,000	—	72	32 } 29	40
			33 }	
			22 }	
Staph. 30,000	18 } 29 } 18 } 30 } 26 }	24	8 } 8	33
			6 }	
Staph. 100,000	—	7	5 } 6	86
			6 }	
			7 }	

Similar experiment and technique as above, 4.12.30.

Staph. 10,000	91 } 81 } 103 } 93 } 78 }	89	6 } 3 } 12 } 6½ 4 }	7
Staph. 20,000	—	44	8 } 6 } 6½ 8 } 4 }	14.8
Staph. 40,000	20 } 14 } 24 } 32 }	22½	4 } 5 } 6½ 2 } 15 }	29
Staph. 80,000	—	11	1 } 6 } 3½ 3 } 5 }	34
Staph. 160,000	—	6	3 } 3 } 2½ 2 } 1 }	37.5

We pass now to other experiments in which we employ, as before, graduated quanta of microbes, and operate again upon blood or separated leucocytes ; but we now arrange our experiments so that the acquirement of bactericidal power of the serum and the effect exerted by it shall be separately manifest.

The simplest and most obvious way of achieving this is to bring the vaccine of dead or living microbes into operation upon the blood, to incubate the clots, and then pipette off the serum and test it by implanting it with *very small* numbers of living microbes.

The two following experiments are of this kind :—

Expt. 17.12.30. A. E. W.'s blood.—5 c.mm. of salt solution or diluted staphylococcus culture were incorporated into 50 c.mm. volumes of blood, and the bloods were then incubated for 20 minutes. After this they were centrifuged

and the sera pipetted off and tested by the Wash and After-wash method—the forerunner volume being here a 10 c.mm. volume of $\frac{\text{Staph.}}{1000}$ and the after-runner volumes three 5 c.mm. volumes of serum. The results were as shown in Table XII.

TABLE XII.

—	No. of colonies which developed in the three after-runner volumes.			Total.
	1	2	3	
Serum from control blood ..	27	20	13	60
Serum from blood implanted with—	26	27	15	68 } 64
320 staph. per c.cm. ..	30	25	13	68
640 staph. per c.cm.	30	24	1	55
1280 staph. per c.cm. ..	23	16	10	49
2560 staph. per c.cm. ..	18	12	0	30

Calculation shows that here, in the most favourable case, the 15 c.mm. of serum here used acquired sufficient bactericidal power to kill at the rate of 2500 staphylococci per c.c.

Expt. 2.12.30. A. E. W.'s blood.—50 c.mm. volumes of blood centrifuged after clotting. The sera were drawn off and were, after addition of Tuberculin B.E., reimposed upon the clots. After 24 hours' incubation the sera were a second time drawn off, and were tested by the Wash and Afterwash method, using a 5 c.mm. volume of $\frac{\text{Staph.}}{1000}$ as a forerunner and three 5 c.mm. volumes of serum as after-runners. (Table XIII.)

TABLE XIII.

—	No. of colonies which developed in the three after-runner volumes.			Total.
	1	2	3	
Control normal serum ..	24	15	4	43
Serum containing—	34	13	6	53 } 48
B.E.	13	7	4	24
2000 million ..	21	6	4	31
B.E.	17	9	0	26
1000 million ..	14	8	0	22
B.E.	18	3	2	23
500 million ..	22	12	3	37
B.E.	13	2	0	15
100 million ..	9	4	4	17
B.E.	15	8	5	28
50 million ..	16	5	0	21
B.E.	8	10	7	25
25 million ..	9	3	2	14

Calculation shows that here again in the most favourable cases the serum acquires sufficient bactericidal power to kill just over 2000 staphylococci per c.cm.

Expt. 21.3.30.—100 c.mm. volumes of A. E. W.'s uncoagulated blood implanted with 10 c.mm. of normal salt solution or staphylococcus culture were incubated for 25 minutes, and then centrifuged. The sera were now pipetted off. 5 c.mm. volumes of a 50,000-fold diluted staphylococcus culture were added in each case to 40 c.mm. of serum. (Table XIV.)

TABLE XIV.

—			No. of colonies which developed.
Serum of control blood	..	..	51
		(1)	50
Serum from blood vaccinated with—		(2)	
5 staph. per c.c.	..	..	39
50 staph. per c.c.	..	..	15
500 staph. per c.c.	..	..	22
1,000 staph. per c.c.	..	..	17
2,000 staph. per c.c.	..	..	17
5,000 staph. per c.c.	..	..	12
20,000 staph. per c.c.	..	..	26

The serum here killed off, at the best, 950 staphylococci per c.cm.

Another way of studying the effect of the serum separately is illustrated by the next two experiments.

Expt. 9.12.30. A. E. W.'s blood.—50 c.mm. volumes of blood centrifuged after clotting in capillary tubes. The sera were then drawn off, 2.5 c.mm. of diluted staphylococcus culture were added to each volume of serum, and the sera were then replaced on the clots and lightly centrifuged. The containing tubes were then disposed almost horizontally and incubated overnight. (Table XV.)

TABLE XV.

Dilution of staphylococcus culture implanted.	No. of microbes implanted into each volume of serum.	No. of colonies which developed.	Percentage of survivors.
Staph. 160,000	6	6 } 5	84
Staph. 80,000	12	2 } 4	32
Staph. 40,000	25	6 } 13	48
Staph. 20,000	50	17 } 20	40
Staph. 10,000	100	21 } 38	38

Calculation shows that here the serum in the most favourable case killed 2500 staphylococci per c.cm.

Expt. 23.9.30. A. E. W.'s blood.—50 c.mm. volumes were filled into capillary tubes and allowed to clot. They were then centrifuged and the sera pipetted off. The volumes of sera were then inoculated with Tuberculin B.E. and then implanted in each case with 58 staphylococci and replaced on the clots. The containing tubes were then sloped so as to form a small angle with the horizontal and were incubated in that position. The number of colonies which developed in the sera were then enumerated. (Table XVI.).

TABLE XVI.

—				No. of colonies which developed.
Control serum	{ (a)			52
	{ (b)			52 } 52
Serum containing—				
B.E.				51
2400 million				47 } 49
B.E.				42
1200 million				41 } 41½
B.E.				47
600 million				44 } 45½
B.E.				35
300 million				37 } 36

Calculation shows that the serum killed 640 staphylococci per c.cm.

Yet another way of arranging the experiment (there is also a fourth, which will be considered elsewhere) is as follows:—

We take a series of capillary tubes upon the stems of which we have marked off two segments of, let us say, 25 c.mm. capacity. We then fill into the distal segments blood from the finger, incubate for 20 to 30 minutes and then expel the clots. Having thus provided ourselves with capillary tubes divided into two segments, of which the distal one is carpeted with leucocytes and the proximal one uncarpeted, we introduce into these combined segments through the proximal ends of the tubes in each case a 50 c.mm. volume of serum—the sera employed for the purpose having been implanted with graduated quanta of staphylococcus. The tubes are now sealed up without displacing the columns of serum, and, the unlined segments being kept uppermost, are sloped so as to form a small angle with the horizontal; they are then placed in the incubator. The protocols of two experiments of this kind are subjoined.

Expt. 2.1.31. R. M. F.'s blood. (Table XVII.)

TABLE XVII.

Dilution of staphylococcus culture implanted into the serum.	No. of microbes suspended in the serum in the		No. of colonies which developed in the serum in the		Percentage of survivors in unlined segment.
	Lined segment	Unlined segment	Lined segment	Unlined segment	
Staph. 180,000	12	12	0 0	15 7 } 11	90
Staph. 90,000	24	24	0 0	7 5 } 6	25
Staph. 30,000	72	72	0 0	33 34 } 33½	48
Staph. 10,000	216	216	0 0	73 82 } 77	30
Staph. 3,000	720	720	0 0	120 120 } 120	17

Here the serum in the unlined segment of the tubes killed in the most favourable case, 24,000 staphylococci per c.cm.

Experiment. A. E. W.'s blood. (Same technique as in the above.) (Table XVIII.)

TABLE XVIII.

Dilution of staphylococcus culture implanted into the serum.	No. of microbes suspended in the serum in the		No. of colonies which developed in the serum in the		Percentage of survivors in unlined segment.
	Lined segment	Unlined segment	Lined segment	Unlined segment	
Staph. 160,000	6	6	0 0	0 3 } 1.5	—
Staph. 80,000	12	12	0 0	12 9 } 10.5	87
Staph. 40,000	24	24	0 0	19 19 } 19	80
Staph. 20,000	48	48	0 0	38 34 } 36	75
Staph. 10,000	96	96	0 0	50 67 } 58	60
Staph. 5,000	192	192	0 0	73 70 } 71½	37

Here the serum in the unlined segment of the tubes killed in the most favourable case, 4800 staphylococci per c.cm.

When we review these experiments we recognise that *the law*—for we may now call it so—*of zero, or all but zero, bactericidal response to minimal microbic*

implantations, and of progressively greater response to increasing microbial stimulation, holds not only of implantations into blood, but also of those made into leucocytic suspensions. And we have further seen that precisely similar effects are obtained with sera pipetted off from respectively lightly and heavily implanted bloods, and also with sera imposed upon respectively lightly and heavily vaccinated leucocytes.

The problem as to whether the increasing killing of serophytic microbes witnessed in effectively vaccinated blood is attributable to increased phagocytic response, or to a development of bactericidal powers in the serum, would appear herein to find its answer.

We shall have to consider later whether the increased phagocytic response which is concurrently obtained does or does not in cases where the action of phagocytes is not mechanically barred, contribute its quota to the observed result.

We may now, taking our cue from the experiments set out in Tables XIII. and XVI., dispose of one further general point before entering deeper into the question of phagocytic response in vitro. On studying the protocols in question you will note two outstanding points of difference between these and those previously considered. It will be noted, first, that instead of the vaccine being directly incorporated into the blood it was here merely placed in abutment against the blood clots. And it will be noted further, that the smaller and larger doses of vaccine here brought into application do not here, as they do when incorporated into the blood, produce opposite effects in the form of positive and negative responses. Instead of that we have here with an extensive range of doses (in the one case with doses ranging from 1 in 2000 million to 1 in 25 million, and in the other case with doses ranging from 1 in 2400 million to 1 in 300 million of Tuberculin B.E.) with every dose a positive bactericidal response.

There is not in reality any matter for surprise in this. For consideration will show that when a vaccine is merely superposed upon a clot there will, in the initial stages of the experiment, come into operation upon the leucocytes only such fraction of the total antigen as may diffuse into the clots. And in conformity with this there will, in the stage of the experiment we are considering, come into lethal operation upon the microbes disposed outside the clot, only the bactericidal substances elaborated in response to this first instalment of antigen.

A number of theoretically interesting points connected with this mode of bringing vaccines into application must be reserved for treatment in a separate paper, but it is of practical interest to reflect that the two contrasted methods of applying a vaccine which we have here been considering have their analogues, the one in intravenous administration and the other in subcutaneous application of vaccines. In the case of the first there is in respect to dosage very little margin of latitude. If we inject a little too much we produce an immediate negative phase. Contrariwise, where a vaccine is applied hypodermically the patient may, even in despite of the administration of an excessive dose, reap benefit from it. For the first instalment of antigen absorbed may achieve the therapeutic aim we have in view, and our aim once achieved any negative phase which may thereafter supervene would be quite innocuous.

INCREASED PHAGOCYTTIC RESPONSE IN VITRO.

We may now pass to the consideration of the phagocytic response obtained in vitro.

TABLE XIX.

A = Percentage of leucocytes which phagocytose.
B = Percentage of leucocytes containing an uncountable number of staph.

—	Phagocytic.		A	B
	Count.	Index		
Heated serum + corp's + $\frac{\text{staph.}}{6}$ + 1 Wash of normal salt	2 3.3	2.6	42	5
Heated serum + corp's + $\frac{\text{staph.}}{6}$ + 1 Wash (= 1/100th) of $\frac{\text{B.E.}}{256 \text{ million}}$			57	5
" " " $\frac{\text{B.E.}}{128 \text{ million}}$	6	2.3	58	16
" " " $\frac{\text{B.E.}}{64 \text{ million}}$	2.6	1	10	8
" " " $\frac{\text{B.E.}}{32 \text{ million}}$	7.7	3	67	21
" " " $\frac{\text{B.E.}}{16 \text{ million}}$	10	3.9	66	19
" " " $\frac{\text{B.E.}}{8 \text{ million}}$	7.1	2.8	60	21
" " " $\frac{\text{B.E.}}{4 \text{ million}}$	6.1	2.3	49	21
" " " $\frac{\text{B.E.}}{2 \text{ million}}$	12.0	4.6	88	48
" " " $\frac{\text{B.E.}}{2 \text{ million}}$	5.6	2.2	56	18

Perhaps when all things are taken into consideration the most dramatic demonstrations of increased

phagocytic response obtained *in vitro* by vaccines are those which are obtained by the use of employing in our phagocytic mixtures instead of unheated serum, heated serum—i.e., serum which is almost denuded of opsonin. It will suffice here to cite a single experiment.

Expt. 5.5.30. A. E. W.'s defibrinated blood.—The blood was centrifuged. The serum was then pipetted off and heated for 45° to 60° C. The corpuscles were washed several times in normal salt solutions. Two volumes of heated serum were then mixed with one volume of washed corpuscles and one volume of a six-fold diluted staphylococcus broth culture, and there was then added to the control phagocytic mixture one wash of physiological salt solution, and to the other mixtures in each case one wash of a dilution of Tuberculin B.E. These mixtures were then, after incubation for 20 minutes, centrifuged and recentrifuged for 45° in each direction. Phagocytic films were then made from each sample of blood. (Table XIX.)

In order to appraise at their proper value the figures set down in the first two columns of the above protocol account has to be taken of the fact that in counting the films any ingest of over 20 staphylococci was entered as an ingest of 20. If it had been possible to count the full number of microbes ingested (the percentage of leucocytes containing uncountable numbers is recorded in column 4 of the protocol) the phagocytic indices would have come out very much higher. The phagocytic counts with the optimum additions of vaccine would certainly have reached astonishing figures—let us say an average of 30 or 40 per cell, and the phagocytic indices would have reached 7.5 or 10.

An experiment like the foregoing furnishes conclusive evidence that leucocytes when treated with vaccines, respond, not only (as seen in previous experiments) by excreting bactericidal substances into the serum, but also by an increased phagocytosis. Similar, but owing to the larger phagocytic intake in the control, quantitatively less striking differences between the vaccinated bloods and the controls are obtained by adding appropriate quanta of vaccines to phagocytic mixtures containing, instead of heated, unheated serum. And analysis of these results by the chiasitic method has shown that increased phagocytic response obtained is, in most cases, due to a combination of increased opsonic power developed in the serum and to increased phagocytic avidity in the leucocytes. Not infrequently, however, the opsonic power of the serum is increased, while the phagocytic avidity of the leucocytes is reduced; and again, sometimes the phagocytic avidity of the

leucocytes is increased while the opsonic power of the serum remains the same or is reduced.

There is, however, in these respects, very little agreement between one experiment and another, and very little also between the figures which represent the phagocytic index of a blood and its component factors—i.e., the figures which represent (a) the opsonic index of the serum, and (b) the phagocytic avidity of the leucocytes. This is no doubt due to the fact that the washing, centrifuging, and standing in salt solution to which the leucocytes are exposed in chiasitic and opsonic experiments all affect their intake, and it is probably also due to the serum of vaccinated bloods containing, in addition to opsonins, antigens which can evoke immunising response in the leucocytes used for testing. There is at this stage no need, I think, to labour the point that the leucocytes of a phagocytic mixture are responsive elements, and are not, in any sense, the analogues of chemical indicators.

IMPORTANCE OF THE FACTOR OF PHAGOCYTOSIS.

I now pass to consider certain general problems which require to be carefully pondered.

The first of these is the problem—already envisaged above—as to whether enhanced phagocytosis or the development of bactericidal power in the blood-fluids is the more important factor in the immunising response which eventuates in increased killing of serophytic microbes in the blood. In connexion with this arithmetical considerations make clear that inasmuch as the blood normally contains $7\frac{1}{2}$ millions of leucocytes per c.cm. there would, if we implanted 100 microbes (and 100 microbes per c.cm. represents a formidable septicaemic infection) be an army of 75,000 leucocytes available for the phagocytosing of each microbe. The same sort of considerations apply to the opsonic capacity of the normal blood fluids. That opsonic capacity as measured (rightly or wrongly) by the ordinary phagocytic technique¹⁴ easily permits of ingests of 10 to 20 microbes per leucocyte of normal phagocytic avidity—and to a total of 75 and 150 millions of microbes ingested per c.cm.

It will be obvious from these data that the normal blood already possesses a phagocytic capacity far in excess of all conceivable requirements. It follows that the explanation of inadequate phagocytosis of

¹⁴ It will be shown elsewhere that quite different results are obtained when we employ (and the centrifugalisation method makes this possible) plasma instead of serum in our opsonic measurements.

implanted or infecting microbes must be sought in the difficulty of leucocytic access to the implanted microbes. When we meditate upon these things and reflect that chemical access (that is access of dissolved bactericidal substances) to implanted microbes must, in the nature of things, be more easily realisable than leucocytic access, and consider further that such chemical access is, in the case of the circulating blood, secured, it comes home to us convincingly that the development of an even moderate amount of bactericidal power in the serum must count for more in the killing of serophytic microbes than any reinforcement of phagocytosis.

The further questions we have to consider are : (1) the question of immediate (I have before called it "*subitaneous*") immunising response ; (2) the question of specific or non-specific immunity (we shall see that specific and non-specific are ambiguous terms) ; and lastly (3) the question of dosage.

THE IMMEDIATE RESPONSE.

One of the things I had to do in connexion with antityphoid inoculation was to refute with data obtained by measuring the bactericidal power of inoculated men before and after inoculation, the traditional belief that ten days must go over before the effect of a vaccination process becomes manifest. I showed there that the effect of inoculation upon the blood was already plainly manifest 24 hours after the administration of the vaccine. A further scaling down of the time deemed requisite for the manifestation of the effects of inoculation was achieved when it emerged from our measurements of the opsonic index that the effects of subcutaneous inoculation and after auto-inoculation were already well developed within an hour after the application of the ictus immunisatorius. And further research has shown that immunising response to vaccines is, in the case of intravenous inoculations, well developed within 10 minutes after the inoculation, and finally, both bactericidal and phagocytic experiments on the blood in vitro—which, of course, lend themselves better to accurate timing—have shown that there is instantaneous response to the introduction of vaccines into the blood. You will, when we come to consider the question of dosage, see that the time requisite for the development of maximum immunising response is not a simple function of the quantum of antigen incorporated, but is largely determined by the soluble or insoluble nature of the antigen

and the lytic forces which the blood brings to bear upon it.

Specific and Non-specific Immunity.

It has already been indicated above that the terms "specific" and "non-specific" are terms of ambiguous signification. Let me justify this.

When we speak of "specific" immunising response, we may mean that infection or its equivalent—i.e., the inoculation of a vaccine—renders the blood bactericidal to the homologous microbes, and only to these; and when we attach this meaning to "specific immunising response" we should, of course, mean by "non-specific immunising response" the acquirement of bactericidal properties for heterologous, as well as homologous microbes.

We may, however, when we use the terms "specific" and "non-specific" be thinking not of the question as to whether immunising response gives protection against one or more varieties of microbes, but of the question as to whether the antibacterial substances brought into play operate "specifically"—each affecting only one species of microbe, or whether they operate "non-specifically"—each affecting more than one species.

Having realised that the terms "specific" and "non-specific" are terms which carry these two distinct meanings, it will avoid confusion if we call the thesis that infection and vaccination immunise only against homologous microbes: "*the doctrine of purely homologous immunising response*," and the antithetic thesis that protection is acquired also against heterologous microbes: "*the doctrine of conjointly homologous and heterologous immunising response*." Similarly, we should do well to call the thesis that there are special antibacterial substances for each kind of microbe: "*the doctrine of specific antibodies*"; and the antithetic thesis that there are antibacterial substances which operate upon more than one kind of microbe: "*the doctrine of non-specific antibodies*."

IS THE IMMUNISING RESPONSE SPECIFIC?

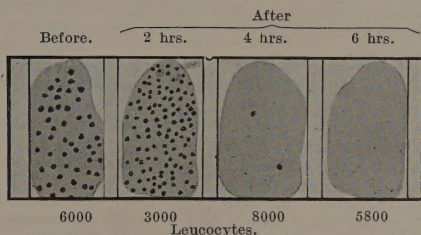
Having clarified the issues we may now make inquiry from the facts, first, as to whether immunising response is specific or non-specific, and secondly, as to whether the antibodies which are native to the blood or are engendered there by infection and vaccination, are specific or non-specific. The answer to the former question is given in many of the protocols of immunisation in vitro already adduced or to be adduced later, as well as in numerous others published

and unpublished. A study of the protocols referred to shows that Tuberculin B.E. gives immunising response upon staphylococcus; that staphylococcus vaccine gives immunising response upon streptococcus, and typhoid vaccine operates in the same way upon staphylococcus. Further, it would seem—at any rate in the case of my own blood—that better immunising response is obtained upon staphylococcus with Tuberculin B.E. than with staphylococcus vaccine.

I shall presently have something to say with respect to this last finding.

When we pass from immunising operations *in vitro* to immunising operations *in vivo* we again find clear evidence of “non-specific”—i.e., heterologous response. Fig. 4 gives a very convincing

FIG. 4.



Coli vaccine, 200 millions (intravenously). Tested in a slide cell by implanting 225 streptococci into 50 c.mm. of blood.

example of response obtained upon staphylococcus by an intravenous inoculation of a coliform vaccine. We have here first a negative phase of diminished and then a positive phase of increased hæmobactericidal action exerted upon the staphylococcus.

And let me, in this connexion, call attention to two points. The first is a question of terminology. It relates to the fact that severe and rapidly supervening constitutional symptoms, such as follow intravenous inoculation, and followed it here, are in the current jargon of medicine, designated “protein shock.” This terminology is to be deprecated because there attach to this term quite erroneous implications. (1) The term suggests that in the case where vaccines produce protein shock this is produced by active agents other than those which produce ordinary immunising response. (2) The term further suggests—for it is employed only in connexion with intravenous injections—that protein shock is produced

only by intravenous inoculations. Those who have administered large doses of typhoid vaccine hypodermically, and have witnessed the resulting supervention of shock in patients, and in particular in patients who have immediately after inoculation engaged in active exercise, will not require to be told that shock can follow when a severely toxic dose of a vaccine given by the subcutaneous channel has been carried into the circulating blood. Another vice of the term "protein shock" lies in its assimilating intravenous inoculations of vaccine to intravenous inoculations of milk, foreign serum, and such like, in lieu of assimilating these injections of foreign albuminous substances to inoculations of vaccine. And an even more serious vice is that the term "protein shock" carries with it the suggestion that non-specific immunising response is a product peculiar to intravenous injections of foreign proteins when it is, in point of fact, a general property of vaccines.

A final point to which I want to draw attention relates to the leucocytic counts inserted at the foot of Fig. 4. These figures illustrate the point that inoculations of vaccine, and in particular, severe inoculations, such as that here in question, are followed by a phase of leucopenia, followed by a phase of leucocytosis; and the figures are inserted here to bring out the point that the increased bactericidal effect shown in the fourth compartment of the slide cell is achieved quite independently of any leucocytosis.

Immunising response *in vivo* is, I need now hardly say, not confined to the case of coliform vaccines. We have evidence that streptococcus gives immunising response *in vivo* upon staphylococcus, and similarly, staphylococcus vaccine upon streptococcus. And these are results obtained by measurements of phagocytic and of sero-bactericidal response—in other words, measurements which cannot be falsified by variations in the number of circulating leucocytes. Let me defer for a moment what I have to say about the importance of these findings for vaccine therapy.

For there can be set off against the protocols of experiments showing non-specific immunising response others which favour the idea that immunising response is specific. I am thinking here of the experiments set out in Charts 32 and 44¹⁵ reproduced in my "Studies on Immunisation" (pp. 418 and 425), and I have in view also some unpublished experiments,

¹⁵ These charts should, however, be studied in conjunction with Charts 7 and 8, pp. 387 and 388, *loc. cit.*

and among these a peculiarly striking one made by Dr. John Freeman. In the experiments in question (they were, in each case, massage experiments) the patients' bloods were tested to two microbes (in Dr. Freeman's case the blood was tested to five), and immunising response was obtained in each case only to that microbe which was from the clinical and bacteriological evidence adjudged to be causally associated with the patient's malady. But obviously, we have no reason to suppose that every species of microbe can immunise against every other species.

ARE THE ANTIBODIES SPECIFIC ?

The study of the question as to whether the antibodies which are native to the blood and also those engendered by inoculation operate upon one kind of microbe to the exclusion of all others has, for the immunisator, only a subordinate interest. A few points may, however, here be referred to. W. Bulloch and G. T. Western,¹⁶ dealing with antibodies native to the blood, found that the opsonins which operate upon the tubercle bacillus can be removed from the serum without removing the opsonins for staphylococcus and vice versa. They found, further, that the opsonin for staphylococcus can be removed without removing the opsonin for the *Bacillus pyocyaneus*. On the other hand, it has been shown (the data will be found in my "Studies on Immunisation," pp. 50 and 51) that treatment of the serum with dead cultures of typhoid and cholera extracts the bactericidins for both species of microbe.

Let me now supplement the points already brought forward by one more. The work of Shattock and Dudgeon—it was, it will be remembered, carried out with melanin particles—suggested that "phagocytic avidity" was non-specific. This conclusion is confirmed by the observations of my sometime fellow-worker, Dr. R. Hare, dealing with the bloods of cases of puerperal septicæmia. These observations show that increased and diminished phagocytic avidity to streptococcus goes hand in hand with increased and diminished phagocytic avidity to staphylococcus.

GENERAL CONCLUSIONS.

I should now like to set out the conclusions which seem to me to follow from the data which we have been considering.

¹⁶ Proc. Roy. Soc. B, 1906, lxxvii.; reprinted in my Studies on Immunisation, pp. 180–185.

Doubtless it seemed paradoxical, and, therefore, doubtful and unacceptable, when I said that when I operate upon my blood with a tubercle vaccine (B.E.) I get better—that is, more emphatic, and for the same dose employed more consistent—immunising response than when I operate with staphylococcus vaccine. In connexion with it I am not going to be rash enough to suggest that the reader should abate anything of his faith in its being a general law of nature for the best immunising response to be obtained by the use of a homologous vaccine. I do not want to do anything more than suggest that a rule such as this might quite well be open to exception. It might be open to a general exception if the heterologous vaccine in question were, as compared with the homologous vaccine, more easily soluble in the blood fluids. And it might be open to an individual exception if a particular patient's blood exerted less lytic action upon a vaccine made with his own microbe than it did upon a vaccine made from a microbe other than the infecting microbe.

Anyone who goes as far as to accept the possibility of such exceptions to rule will be prepared to let the superiority of a vaccine A over a vaccine B—even if the vaccine A should be heterologous and the vaccine B homologous—be decided by laboratory experiment and, moreover, will be prepared to look upon such experiments without *parti pris*. But while I would plead that the question of superiority of one kind of vaccine over another is one that can be settled only by experiment, none the less I want to put two general points. They are the following:—

In cases where the nature of the infecting agent cannot be determined, advantage very often accrues from inoculation with vaccines made from microbes which are, in all probability, different from those with which the patient is infected. When I say this I have in view (well knowing that these and all other bacteriologically undiagnosed affections are of debatable ætiology) articular rheumatism and phlyctenules of the cornea. It would, I think, be sheer obstinacy to let doubt respecting the ætiology of these affections and a passionate conviction that immunising response is purely specific, hold us back from inoculating in the one case vaccines made from intestinal streptococci, and in the other case minimal doses of Tuberculin B.E. Essentially the same reasoning would, I think, apply in dealing with infections such as glanders, in which homologous vaccines have been tried time and again without success. One might, I think, surmise that such a

microbe as that of glanders furnished a very ineffective antigen, and one might, by consequence, reasonably try whether anything useful could be done with a non-homologous vaccine.

Principles of Dosage.

I turn now—and this will complete what I propose to say here on the subject of vaccine therapy—to the question as to whether we can, with the knowledge gained by the study of immunisation in vitro, add anything to the principles of dosage which were learned when we had only the clinical symptoms and measurements of the opsonic index to guide us. It will be remembered that the most important of the general principles of dosage arrived at by watching and measuring the opsonic index of patients was that the doses of vaccine employed should be inversely as the amount of infection. Further, that, with a view to avoiding negative phases, the doses given to heavily infected patients should be much smaller than those given to the lightly infected, and should represent a very small fraction of the doses administered in prophylactic operations undertaken upon the healthy. This principle finds interesting confirmation when we carry out experiments in vitro. We take a series of equal measured blood volumes and implant into these graduated quanta of living microbes, and then add to each implanted volume one and the same dose of vaccine. It does not require a great effort of imaginative vision to discern that we have here analogous conditions to those which prevail in lightly and heavily infected patients. Instructive illustration of the effects produced is furnished in the subjoined protocols.

Expt.—Capillary tubes were prepared with a stem about a foot and a half in length and of almost uniform bore, and a 10 c.mm. division mark was then placed on the distal end of the stem. Blood was then obtained from my finger, and then there was aspirated into one of the capillary tubes as a forerunner 10 c.mm. of a 1000-fold dilution of a broth culture of staphylococcus; and then, as after runners, ten 10 c.mm. volumes of blood. The same procedure was carried out with two other portions of blood, the one inoculated with a smaller and the other with a larger dose of Tuberculin B.E. After 24 hours' incubation the blood-clots were blown out in order, each series arranged in a row upon a separate strip of filter paper. The clots were then, as soon as they had by absorption of fluid become adherent to the filter paper strips, treated with 1 per cent. saponin, and the strips were then introduced into a beaker full of water, being applied to its interior wall after the fashion of a cornice. When hæmolysis was complete the filter paper strips were placed in a Petri dish containing water, and the decolorised

clots were then aspirated into a capillary tube and transferred in proper order to a microscopic slide. They were then dried and stained. The number of colonies which developed in each of the three series of clots is set out in Table XX.

TABLE XX.

	No. of colonies of staphylococcus in volumes.										No. of colonies in—		
	1	2	3	4	5	6	7	8	9	10	The whole 10 vols.	Vols. 1-5	Vols. 6-10
Control blood ..	1	9	5	2	1	2	1	1	0	0	22	18	4
Blood inoculated with B.E. 1 in 1000 million ..	1	3	4	1	2	0	2	0	0	0	13	11	2
Blood inoculated with B.E. 1 in 500 million ..	4	10	9	15	3	1	0	0	0	0	42	41	1

Note.—The small number of colonies in Vols. No. 1 is no doubt due to this volume of blood being very highly diluted by the forerunner volume of tuberculin in salt solution.

When we glance, to begin with, at the rubric in which are set down the aggregate number of staphylococci colonies which developed in the three capillary tubes, we see that the aggregate is, in the case of the blood containing 1 part of B.E. in 1000 million, less by nearly 50 per cent. than it is in the control blood, and that the aggregate number of colonies which developed in the blood which contained 1 part of B.E. in 500 million is nearly double that in the control. If demonstration were necessary of the impossibility of judging a dose of vaccine by the epitomal result produced upon a series of different bloods of patients, such demonstration is furnished here. For, judged by the epitomal effect produced in the whole series of volumes, a dose of 1 part in 500 millions of tuberculin is a large overdose for my blood. Very different are the conclusions which emerge when we analyse the results and consider separately that which applies to the first five and that which applies to the last five volumes of the series. We then see that in the case of the heavily implanted blood volumes the effect of the larger dose of vaccine is to give us in these volumes more than twice as many colonies as in the control blood, and nearly four times as many as in the less heavily vaccinated blood. But when we turn to the effect produced upon the more lightly implanted bloods we see that the larger dose of vaccine enhances the bactericidal power more than does the smaller dose.

An essential similar experiment is Table XXI.

Expt. 8.4.31.—Same technique. Forerunner 15 c.mm. volumes of staph. ¹⁰⁰⁰; afterrunners, ten 15 c.mm. volumes of A. E. W.'S blood.

TABLE XXI.

—	No. of staphylo- cocci colonies in volumes.*									Whole 8 vols.	1st 4 of those vols.	2nd 4 of those vols.
	3	4	5	6	7	8	9	10				
Control—												
Unvaccinated blood	24	16	9	3	0	0	1	1	54	50	52	2
	29	12	2	3	0	0	0	0	46		46	
	20	9	20	0	4	1	0	0	54		49	
	29	7	3	3	0	3	0	0	45		42	
Blood vacc. with—												
B.E.												
1000 million ..	2	0	2	1	0	1	0	0	6		5	1
B.E.												
500 million ..	11	8	5	0	1	0	0	0	25		24	1
2000 killed staph.	15	4	6	2	0	0	1	0	28		27	1
4000 ,, ,,	23	14	14	14	4	0	0	0	69		65	4

* Nos. 1 and 2 nil. Staph. = staphylococci.

Increased Phagocytosis Obtained by Inoculation of Blood in vitro.

Precisely similar effects—and effects which show that we are here dealing with a general law—are seen in phagocytic experiments in which an inoculated blood is tested with progressive dilutions of a staphylococcus culture.

Phagocytic Expt.—A. E. W.'s blood centrifugalised with different strengths of Tuberculin B.E. and different strengths of staphylococcus vaccine. (Table XXII.)

TABLE XXII.

—	Phagocytic indices tested with—	
	5-fold diluted sterilised culture.	15-fold diluted sterilised culture.
Control blood	1.0	1.0
Blood with—		
B.E.		
1000 million	0.76	1.63
B.E.		
200 million	0.66	1.0

No preliminary incubation.

Phagocytic Expt.—A. E. W.'s defibrinated blood mixed with different strengths of Tuberculin B.E. and different dilutions of staphylococcus and then centrifugalised immediately after mixture. (Table XXIII.)

TABLE XXIII.

		Phagocytic indices tested with—			
		Undiluted culture.	3-fold dilution.	9-fold dilution.	27-fold dilution.
Control blood	..	1.0	1.0	1.0	1.0
Blood with—					
B.E.	..	1.5	2.45	1.65	1.2
1000 million					
B.E.	..	1.4	1.0	1.0	0.96
5000 million					

Phagocytic Expt.—A. E. W.'s defibrinated blood implanted with Tuberculin B.E. and centrifugalised immediately after mixture with progressive dilutions of staphylococcus. Where the higher dilutions of staphylococcus were employed the length of centrifugalisation was increased in order to obtain a conveniently large ingest. (Table XXIV.)

TABLE XXIV.

		Phagocytic indices tested with—			
		An undiluted culture.	3-fold dilution of the culture.	9-fold dilution of the culture.	27-fold dilution of the culture.
Control blood	..	1.0	1.0	1.0	1.0
B.E.					
Blood with 1000 mill.	..	1.05	1.1	1.35	1.4
Duration of centrifugalisation	..	$\frac{1}{2}$ min.	1 min.	2 min.	2 min.

The rationale of these results is obvious. They show us that when the blood is implanted with a moderate quantum of living microbes and a moderate quantum of vaccine is added, the antigens derived from these twin sources may constitute, taken together, an optimum immunising stimulus. They show us further that when the implant of living microbes is cut down, and the antigen derived from this source is correspondingly diminished, the quantum of vaccine may be profitably increased. And vice versa when the implant of living microbes is increased the quantum of vaccine must be correspondingly cut down.

Before leaving this question a word may be said concerning the situation in which we find ourselves when dealing with a patient who has an acute infection, and we are in doubt as to whether he can still make response to a vaccine, and if so as to what quantum can be safely administered. In this situation we

can, by recourse to what I have called the Vaccine Response Test¹⁷ obtain the required information.

The "*Vaccine Response Test*" consists in adding to the patient's blood in vitro graduated quanta of the vaccine which it is proposed to use, and seeing whether any of the doses employed enhance, after some arbitrarily fixed period of incubation, the phagocytic power of the patient's blood, or whether all doses of vaccine impair that power.

I have elsewhere¹⁸ published data showing the help that can be obtained from the vaccine response test. But I should like to say here that when in a rapidly advancing infection we needs must decide in the dark, it is always advisable to try the effect of inoculating with a very small dose of vaccine. For the advantage that may accrue if the patient's blood is still capable of making immunising response far outweighs the harm that might result if his were incapable of making immunising response, and the case already hopeless.

Let me now come back from this digression and consider yet another question in connexion with the dosage of vaccines. I can best introduce the point by recalling that while in the case of ordinary vaccines some of the antigen is in solution, the bulk of it is present in the form of particulate matter which is (I am thinking here in particular of vaccines made from serophytic microbes) not very readily soluble in the blood fluids. It follows that if there is, when the vaccine comes into operation upon the blood, any immediate immunising response, this will be response to that fraction of the total antigen which is in solution in the vaccine, or is, as the case may be, immediately brought into solution by the blood fluids. Such subitaneous response takes in the typical curve of opsonic response (figured in my "*Studies of Immunisation*," p. 379, Chart I), the form which I there called a "false rise," and this "false rise" is, according as the quantum of antigen afterwards dissolved is appropriate or excessive, followed by either a positive phase unpreceded by a negative, or a negative phase followed by a positive. All this is very clearly shown in the subjoined series of protocols.

Phagocytic Expt.—A. E. W.'s defibrinated blood treated with staphylococcus vaccine and tested with staphylococcus by the method of centrifugalisation at intervals after vaccination. (Table XXV.)

¹⁷ THE LANCET, 1923, i., 473.

¹⁸ THE LANCET, March 10th, 1923.

TABLE XXV.

	Phagocytic indices.		
	Immediate result.	After 1 hr. 45 min. on bench.	After a further 45 min. at 37° C.
Control blood	1.0	1.0	1.0
Blood inoculated with 10,000 dead staph. per c.cm.	1.23	0.55	0.18
Blood inoculated with 5 million dead staph. per c.cm.	1.33	0.77	0.31

Phagocytic Expt.—A. E. W.'s defibrinated blood vaccinated with Tuberculin B.E., and tested at intervals after vaccination by centrifugalisation with staphylococcus. (Table XXVI.)

TABLE XXVI.

	Phagocytic indices.	
	Tested immediately after vaccination.	After 1 hr. 20 min. incubation.
Control blood	1.0	1.0
Blood inoculated with— B.E.		
1000 million	1.5	0.6
B.E.		
200 million	0.57	0.7
B.E.		
10 million	0.53	0.7

TABLE XXVII.

	Phagocytic indices.					
	5 min.	10 min.	20 min.	40 min.	90 min.	140 min.
Control blood	1.0	1.0	1.0	1.0	1.0	1.0
Blood inoculated with— B.E.						
1000 million	0.82	0.94	1.0	1.15	1.63	1.6
B.E.						
50 million	0.6	0.56	1.0	0.8	0.76	0.86

Phagocytic Expt. 22.10.25.—A. E. W.'s blood and the same blood treated with two strengths of Tuberculin B.E. Tested phagocytically by centrifugalisation with staphylococcus at different intervals after vaccination (Table XXVII.)

Expt. 23.10.27.—A. F.'s defibrinated blood implanted with Tuberculin B.E. and kept in the incubator and tested at progressive intervals after vaccination by centrifugalisation with staphylococcus. (Table XXVIII.)

TABLE XXVIII.

	Phagocytic indices.			
	5 min.	10 min.	1 hr.	1 hr. 20 min.
Control blood ..	1.0	1.0	1.0	1.0
Blood inoculated with—				
B.E. ..				
500 million ..	0.89	0.86	1.53	1.64
B.E. ..				
5 million ..	1.25	1.1	1.25	1.0

Expt. (undated).—A. F.'s defibrinated blood incubated with Tuberculin B.E. and tested at progressive intervals after vaccination by centrifugalisation with staphylococcus. (Table XXIX.)

TABLE XXIX.

	Phagocytic indices.				
	10 min.	14 min.	20 min.	25 min.	30 min.
Control blood ..	1.0	1.0	1.0	1.0	1.0
B.E. ..					
Blood with 300 million ..	1.46	1.47	1.31	1.30	0.90

There remains one last point which I should like to call attention to. It is again a point of similarity between the effects produced by infection in vivo and those produced by inoculation in vitro. It will perhaps be remembered that I have from the very outset called attention to the fact that patients suffering from lupus and generally from strictly localised forms of tuberculosis,¹⁹ unaccompanied by constitutional disturbance, have a very low opsonic index to tubercle.

The phenomenon is clearly not one of the same order as the negative phase. For negative phases supervene, not upon minimal auto-inoculations., such as can here come in question, but only upon large inoculations or auto-inoculations. Further, a negative phase is practically always followed by the response in the form of a positive phase, and the indices of these patients remain, as can be inferred from the fact that their tuberculo-opsonic indices are uniformly low, chronically below standard. Every one of these points finds its

¹⁹ The data relating to this question and also important confirmation of these data by Prof. W. Bulloch are to be found in my *Studies of Immunisation*. (pp. 118, 152, and 146-148).

parallel in the results obtained by the inoculation of the blood with minimal quanta of vaccine.

It has already been seen in one of the protocols of hæmobactericidal experiments which I have shown—and I could have very easily produced others showing the same—that when minimal quanta of living microbes are implanted into the blood on the one hand and serum on the other, more microbes develop into colonies in the blood than in the serum. The same thing may be seen when the sera of bloods which have been treated with minimal quanta of vaccines are tested for bactericidal power by implantation with staphylococcus. Such sera are very often found to furnish a better culture medium for staphylococcus than the control sera. And again, the addition of minimal quanta of vaccine to a phagocytic mixture often lowers the phagocytic index.

In conclusion, I have to express my gratitude to my fellow-workers, and in particular Prof. A. Fleming, Dr. Leonard Colebrook, Dr. R. M. Fry, and Dr. R. Mummery, for having very generously placed not only their work, but also their ideas at my disposal.

